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Applicant: Raymond P. Warrell, Jr. *et al.*

Title: *PROCESS FOR PRODUCING ARSENIC TRIOXIDE FORMULATIONS AND METHODS FOR TREATING CANCER USING ARSENIC TRIOXIDE OR MELARSOPROL*

Serial No.: 10/425,785 Filing Date: April 30, 2003

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DECLARATION UNDER 37 CFR §1.132

Commissioner for Patents
PO Box 1450
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10 September 2003

Sir:

I, Ralph Ellison, state that:

1. I am a consultant to Cell Therapeutics, Inc. (CTI), the exclusive licensee of the application in caption ("the application"). CTI acquired its rights in the application through its purchase of PolaRx Biopharmaceuticals, Inc, a company that I co-founded and ran. While at PolaRx I was responsible for all aspects of clinical development of Trisenox® (intravenous arsenic trioxide). In my current capacity as consultant, I work closely with the Trisenox® (intravenous aresenic trioxide) clinical development team. I am familiar with the claims pending in the application.

2. I received my medical degree in 1986 from the University Of The Witwatersrand, in Johannesburg, South Africa. Before the application was filed, I was the head of the Company that developed Trisenox and worked closely with Dr. Ray Warrell, an inventor named in the application who at that time was a hired consultant to PolaRx. PolaRx sponsored the development in the United States of a protocol for treating acute promyelocytic leukemia (APL) via weight-based dosing of arsenic trioxide (ATO).

3. Dosage schemes for cancer treatment generally are quite different than those for the treatment of other diseases. Because cancer typically is caused by an abnormal proliferation of the

patient's own cells, for example, the targeting of disease tissue and cells will be non-specific and, hence, will have a detrimental effect on the patient's healthy tissue and cells. Consequently, toxicities that are unacceptable in the treatment of an allergic reaction, an infectious disease, and many other conditions are considered acceptable in the treatment of cancer, given the often deadly nature of latter condition. A decision to use a potentially toxic drug in cancer therapy is based, therefore, on a risk/benefit analysis.

4. The approach to determining a safe dose for most non-cancer disease types takes into account the size of the patient by weight, and this frequently gives rise to the use of a fixed or "flat" dose for a class of patients, such as all adult patients. This approach eases the calculation of the dosage amount during treatment. In the oncology field, on the other hand, the primary method for balancing the safety and the efficacy of a drug treatment entails metering dose by reference to the surface area of the patient.

5. For example, Smorenburg *et al.* states that, in "medicine, most drugs for adult patients are administered at a flat-fixed dose.....In contrast, in oncology, the dosage of nearly all cytotoxic drugs is based on body-surface area (BSA) of the patient."[1] That is, instead of using a ratio of milligrams of drug per kilogram of patient, the standard approach in cancer treatment couches dosage in terms of milligrams of drug per square meter of patient surface area. This makes for a much more complex calculation of the actual amount to dose during treatment.

6. The prevalence of surface area dosing in cancer therapy is due in part to the potentially complex pharmacokinetics of weight based dosing between species, using conversion factors. By contrast, conventional wisdom in cancer therapy applies a conversion factor of one (1) in relation to surface area-dosing pharmacokinetics between species. See Voisin *et al.*[2] and Freireich *et al.*[3] This simplifies the assessment of a proposed safe dose in a field where the balance of efficacy to toxicity can be very problematic, and BSA dosing therefore has become the gold standard in oncology. As recently as 2001, Gurney notes that, "until there is a better method, BSA-dosing will prevail since there has

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[1] *J. Clin. Oncology*, 21:197-202 (2003).

[2] *Regulatory Toxicology and Pharmacology*, 12:107-116 (1990).

[3] *Cancer Chemotherapy Reports*, 50:219-244 (1966).

been over 40 years of experience with this method and 'old habits die hard.'"[4] Thus, even while debate continues on current, BSA-based dose calculations for chemotherapy, oncologists principally employ BSA dosing to balance toxicity and efficacy based on patient size.

7. Those working in the oncology field sometimes consider flat dosing as an alternative to BSA dosing. For example, Westervelt *et al.* explored the adjusting of a flat dose of arsenic trioxide for a single APL patient.[5] In their 1997 abstract, Westervelt *et al.* flat-dosed the patient 10 mg of arsenic trioxide daily. In view of a resultant and profound leukocytosis, as well as other parameters indicating lack of efficaciousness, Westervelt *et al.* increased the dose to 50 mg/day. Having observed significant toxicity during and after the treatment, they concluded that toxicities had to be considered when dosing arsenic trioxide.[6]

8. In the course of developing a clinical protocol for treatment of APL with ATO, the present inventors also initially adopted a flat-dosing approach and, with their first five patients, used a daily 10-mg dose.[7] Patient 5 in the initial group relapsed within 24 days of achieving total remission and before completion of the consolidation therapy. As the patient was a very large individual (163 kg), the inventors questioned whether he might have received too little drug at a flat dose of 10 mg daily. The relevant literature did not suggest this problem, since there was no teaching that the size of a patient should be considered in arriving at an appropriate dosage.

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[4] *Brit. J. Cancer*, 86:1297-1302 (2001).

[5] Abstract 3859, *Blood*, 90 (Suppl. 1): 249b (1997)

[6] Westervelt *et al.* also back-calculated the administered flat doses, identifying them in weight-based terms, too. Thus, the initial 10 mg/day dose was translated to 0.08 mg/kg, and 50 mg/day dose to 0.40 mg/kg. *Id.* The fact that flat dosing was used for this initial patient is confirmed in a later article by Zhang *et al*, which again references "10 mg daily" and "50 mg daily" for this same patient, while characterizing the protocol for 4 subsequently-treated patients as being one "with the dosage based on actual body weight." In both the abstract and in this later study, it is suggested that full Phase I/II studies would be needed to determine a proper dosing level for a broad population. See Zhang *et al.*, *Modern Pathology*, 13:954-61 (2000). Another later study, reported by Westervelt *et al.*, *Blood*, 98:266-71 (2001), did undertake a dose escalation study in order to determine an appropriate dosage. Only Phase I has been reported, and no dose escalation beyond the initial dose of 0.1 mg/kg per day was undertaken, possibly because of the "2 unexpected deaths" among the first nine patients. Thus, the Phase I trial failed to determine a proper dosing level, and leaves open the question of whether a dose that results in both efficacy and acceptable toxicity can be achieved for this drug.

[7] The flat dosing was in accordance with earlier reports illustrated by Westervelt *et al.* (1997), *supra*, Shen *et al.*, *Blood*, 89:3354-3360 (1997), and Zhang *et al.*, *Chinese J. of Hematology*, 17(2) (1996).

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9. Since the drug had been well tolerated by the initial patients, and in order to avoid the possibility of under dosing, as was believed to have occurred with Patient 5, the dose was increased to a 15 mg flat dose for all subsequent patients. This dosage amount was given to Patients 6 and 7. Patient 8 was a 13 year-old girl and of smaller stature, however. For this patient, therefore, the inventors chose to revert to the original, 10 mg-daily dosage, as a precaution against the possibility of overdosing. Patient 9 was a 9 year-old boy and, because of his size, was given a flat dose of only 5 mg daily. Patient 10 was given the newer dosage of 15 mg daily.

10. Upon reviewing the results for the first ten patients, the inventors concluded that the standard flat dosing method, per Shen and Zhang, appeared not to be efficacious for large people and was too toxic for small people. They further concluded that their initial approach of adjusting the flat dose was arbitrary and did not allow for a balancing of toxicity and efficacy in a treatment protocol to be used across a broad population of patients. Prior to treating Patient 11, therefore, the inventors decided to implement a technique other than flat dosing. Rather than turning to standard BSA dosing, the technique widely used by oncologists for dosing of chemotherapeutic drugs, the inventors chose to attempt to develop a weight-based dosing scheme. Employing data generated from the first ten patients, the inventors calculated a putative weight-based dose of 0.15 mg/kg daily. This dose was used for the next two patients and was ultimately chosen to complete the study and to conduct the pivotal phase III trial in which 40 patients participated. The results of this trial are reported in Soignet *et al.*, *J. Clin. Oncology*, 19:3852-3860, and showed that arsenic trioxide treatment is both safe and effective. Eighty-five percent of patients achieved clinical complete remission, and there were no treatment-related deaths.[8] Westervelt *et al.* contrasts the results achieved in the trial reported in Soignet *et al.* with the unexplained deaths in their study, noting that "in another series of 40 APL patients treated with 0.15 mg/kg per day arsenic trioxide for variable periods, no life-threatening arrhythmias or treatment-related deaths were reported."[9] While proffering various theories to explain the differing results, Westervelt *et al.* reached no conclusion on this point.

11. The results obtained in the trial reported in Soignet *et al.* led to approval by the FDA of arsenic trioxide (Trisenox). Subsequent to FDA approval of Trisenox , data on an additional 2,228

[8] Soignet *et al.* (2001), page 3854 ("clinical efficacy") and page 3856 ("adverse events").

[9] Westervelt *et al.* (2001), page 270.

patients (treated at doses of about 0.15 mg/kg per day or greater) have been collected, via post marketing surveillance and in clinical trials. To date no deaths attributed to arsenic associated arrhythmia have been reported, providing further evidence that treatment with arsenic trioxide is safe.

12. Subsequent to the present invention, another group of oncologists chose to modify flat dosing to a dosing based upon the patient's size; this, in recognition of a need to protect patients from toxic doses of ATO during the APL treatment. See Au *et al.*, *Annals of Oncology*, 14:752-57 (2003). Au *et al.* adopted a BSA dosing scheme, however. Thus, they described the use of BSA dosing in the context of treating a group of patient with relapsed APL. Initial treatment was on a flat-dosage basis for APL patients who underwent bone-marrow transplantation and ATO therapy. For double-relapse patients, however, the dosage was metered to take into account the size of the patient on a surface area basis. The difference in initial dosing and double-relapse dosing can only be interpreted as an acknowledgement of the need to balance toxicity and efficacy for the patients who had been weakened by extensive therapy beforehand. When faced with the same problem that the present inventors confronted, in other words, Au *et al.* resorted to more conventional treatment scheme, with dosing based upon patient surface area.

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I hereby declare that all the statements made herein of my known knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Sept. 11 2003
Date

Ralph Ellison

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Original article

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Arsenic trioxide in comparison with chemotherapy and bone marrow transplantation for the treatment of relapsed acute promyelocytic leukaemia

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Background: The best overall treatment strategy for patients with acute promyelocytic leukaemia (APL) in relapse with chemotherapy, bone marrow transplantation (BMT) or arsenic trioxide (As_2O_3) based therapy remains undefined.
Patients and methods: We reviewed the clinical course and treatment outcome of 143 APL cases seen in four major hospitals in Hong Kong over a 10-year period.
Results: Complete remission (CR) was attained in 113 cases (79%) with all-*trans* retinoic acid (ATRA) and chemotherapy. Relapse occurred at a median of 16 months in 54 cases, with a 3-year disease free survival of 56%. Post-relapse treatment was successful in 41 cases (76%), giving an actuarial 3-year overall survival (OS) of 81% from CR1. Three different protocols were used: chemotherapy alone (n = 19), allogeneic BMT (n = 14) and an As_2O_3-based regimen (n = 21). Chemotherapy was associated with the highest treatment-related mortality (TRM) at 53%, giving a CR2 rate of 47%. TRM was 36% for BMT. The CR2 rate for the As_2O_3-based regimen was 100%, with no TRM. However, 38% of As_2O_3 treated patients had subsequent relapses, which were further salvaged in 75% by combined As_2O_3 plus ATRA. The actuarial OS for the three protocols leveled off by 2 years at 82% for As_2O_3, 43% for BMT and 23% for chemotherapy (P = 0.0004).
Conclusions: Our results suggest that As_2O_3 may be superior to chemotherapy and BMT for the treatment of APL in relapse.
Key words: acute promyelocytic leukaemia, allogeneic bone marrow transplantation, arsenic trioxide, relapse

Introduction

Acute promyelocytic leukaemia (APL) is characterised by t(15; 17)(q22; q21), which results in *PML/RARA* gene fusion. It is highly sensitive to all-*trans* retinoic-acid (ATRA), which acts as a differentiation agent [1], and arsenic trioxide (As_2O_3), which induces both differentiation and apoptosis [2]. Although APL blasts are very sensitive to chemotherapy, particularly anthracyclines [3], induction chemotherapy is associated with early death due mainly to haemorrhagic complications [4]. With the use of ATRA combined with chemotherapy, haemorrhagic complications can largely be avoided. However, up to 20% of patients still relapse, despite the use of chemotherapy and ATRA as consolidation and maintenance treatment [5].

In relapsed APL, the best treatment strategy remains contentious. Some patients are still responsive to ATRA and chemotherapy. Allogeneic bone marrow transplantation (BMT) may give lasting remissions, but the patient selection and timing for BMT are unresolved issues. Clinical and laboratory evidence indicates that As_2O_3 is a highly effective treatment for relapsed APL. However, the role of As_2O_3, in comparison with further chemotherapy or allogeneic BMT, has not hitherto been formally evaluated.

In this report, we studied the treatment results of newly diagnosed and relapsed cases of APL over a 10-year period, with a specific focus on evaluating the relative merits of chemotherapy, allogeneic BMT and As_2O_3 in the treatment of relapses.

Materials and methods

Patients

All patients with APL treated between 1991 and 2001 were included in the analysis. They were treated in four tertiary referral centres (Queen Mary Hospital, Queen Elizabeth Hospital, Tuen Mun Hospital, Pamela Youde Nethersole Hospital) that served over 70% of leukaemia patients in Hong Kong during that time period. The diagnosis of APL was based on marrow

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Table 1. Treatment and outcome of 54 patients with relapsed acute promyelocytic leukaemia

Regimens	n	Early deaths	CR (%)	Second relapse, % (median time)	Outcome of further relapses
Chemotherapy	19	53% (bleeding, sepsis)	47	22 (2/9) at 11 months and 21 months	Both died
BMT	14	36% (mucositis, sepsis)	64	11 (1/9) at 40 months	CR with second BMT
As_2O_3	21	-	100	38 (8/21) at 13 months	Two died, six in CR with As_2O_3 + ATRA

CR, complete remission; BMT, bone marrow transplantation; As_2O_3, arsenic trioxide; ATRA, all-*trans* retinoic acid.

Table 2. Outcome of 14 acute promyelocytic leukaemia patients treated with allogeneic bone marrow transplantation (BMT)

	Sex/age (years)	Time	Status	Conditioning	Engraftment		GvHD	OS	DFS	Complications
					Plt >25	ANC >1				
1	M/22	4.0	R1	BuCy	24	18	0	122.9+	35.4	Relapse, 2nd BMT done
2	F/22	20.4	CR2	BuCyTBI	20	19	2	55.7	55.7	Died of bronchiolitis obliterans
3	F/30	10.3	CR2	BuCyTBI	23	19	3	98.9+	98.9+	cGvHD
4	F/14	17.9	CR2	BuCyTBI	13	17	2	93.5+	93.5+	Hemorhagic cystitis
5	M/39	20.1	R1	BuCyTBI	44	24	4	3.1	3.1	Die of sepsis
6	M/27	6.1	R1	BuCy	34	15	2	85.6+	85.6+	AVN of hip
7	F/12	5.8	R1	BuCy	27	30	2	13.3	13.3	Died of sepsis
8	M/35	17.7	R1	BuCyTBI	24	20	2	6.2	6.2	Died of mucositis
9	M/32	46.1	CR2	BuCyTBI	27	19	2	7.2	7.2	Died of sepsis
10	F/45	16.6	CR2	BuCyTBI	20	24	4	1.4	1.4	Died of liver failure
11	F/14	22.1	CR2	BuCy	18	15	0	64.2+	64.2+	Nil
12	M/45	22.1	CR2	BuCyTBI	14	15	2	9.7	9.7	Died of liver failure
13	F/47	15.7	CR2	BuCyTBI	28	25	2	3.7	3.7	Died of sepsis
14	F/35	19.0	CR2	BuCyTBI	13	18	2	45.5+	45.5+	cGvHD, transient graft failure[a]

M, male; F, female; time, time from initial diagnosis to BMT in months; R, relapse; CR, complete remission; Bu, busulphan; Cy, cyclophosphamide; TBI, total body irradiation; Plt >25, days to platelet count >25 × 10^9/l; ANC >1, days to absolute neutrophil count >1 × 10^9/l; GvHD, acute graft-versus-host disease (grades 0–4); OS, overall survival in months; DFS, disease-free survival in months; +, survivor; cGvHD, chronic graft-versus-host disease; AVN, avascular necrosis of hip.

[a]One month of marrow aplasia with spontaneous recovery due to idiosyncratic hypersensitivity to azathioprine for GvHD.

morphology, and was confirmed by cytogenetic and/or molecular investigations [6].

Treatment of newly diagnosed APL

The standard induction protocol was ATRA (45 mg/m²/day ×6 weeks), together with daunorubicin (50 mg/m²/day ×3 days) and cytosine arabinoside (100 mg/m²/day ×7 days). Consolidation therapy consisted of two to four courses of an anthracycline (daunorubicin or mitoxantrone) containing regimen. Maintenance therapy (ATRA 45 mg/m²/day ×15 every 3 months, methotrexate 15 mg/m²/week, 6-mercaptopurine 50 mg/m²/day for 18 months) was used in three centres. Prospective monitoring of minimal residual leukaemia was not performed routinely.

Treatment of relapsed APL

All relapses were diagnosed by marrow biopsy and confirmed cytogenetically or molecularly (Table 1). From 1991 to 1997, chemotherapy and ATRA were used for induction of second complete remission (CR2) (n = 33). Patients reaching CR2 and with a suitable marrow donor (n = 14) proceeded to allogeneic BMT, while the others (n = 19) received consolidation with conventional chemotherapy. After 1997, As_2O_3 (10 mg daily until remission) and idarurbicin (72 mg/m² in nine divided doses over 3 months) were used in all relapsed cases (n = 21), as previously reported [7]. Patients who relapsed again after As_2O_3/idarubicin treatment (n = 8) were further treated with As_2O_3 (10 mg/m²/day) and ATRA (45 mg/m²/day) until CR3, followed by further consolidation with As_2O_3 plus ATRA, each given for 14 days every 4–6 weeks for six courses, as reported previously [8].

Allogeneic BMT

From 1991 to 1997, all relapsed patients reaching CR2 and with a human leukocyte antigen (HLA)-identical donor (n = 14; 13 from siblings and one from a matched-unrelated donor) were considered suitable for allogeneic BMT (Table 2). There were no exclusion criteria. Conditioning regimen comprised: busulphan (16 mg/kg) and cyclophosphamide (120 mg/kg) in four cases; and busulphan (7 mg/kg), cyclophosphamide (50 mg/kg) and total body irradiation (TBI; 12 Gy) in 10 cases. Melphalan (100 mg/m²) and TBI (12 Gy) was used for the second BMT in one patient (case 1; Table 2). Cyclosporine and methotrexate was used in all cases for graft-versus-host disease (GvHD) prophylaxis.

Statistical analysis

Data were censored on the last day of 2001. For the whole group, actuarial survival was calculated by Kaplan–Meier analysis. Patients with or without

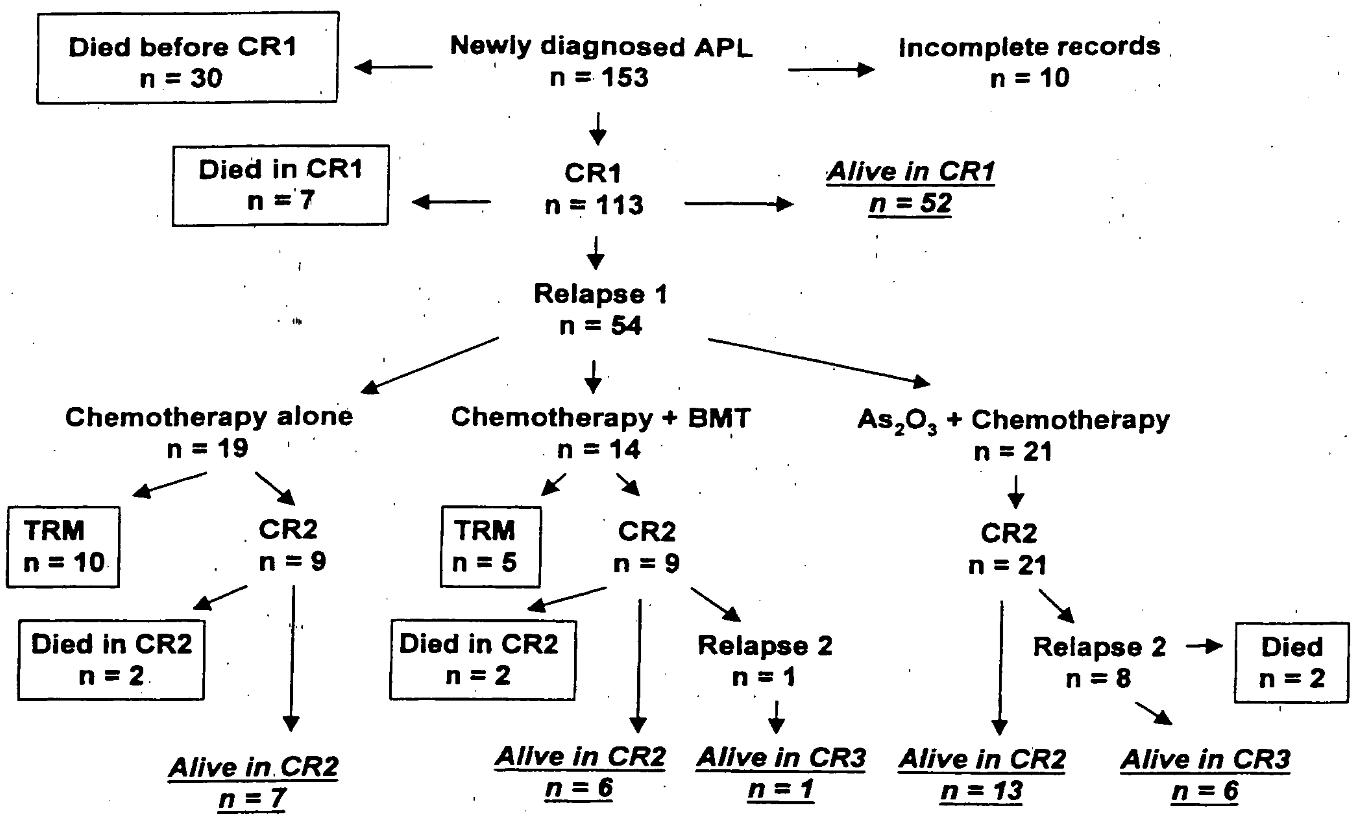

Figure 1. Treatment protocol and outcome of 153 cases of newly diagnosed acute promyelocytic leukaemia. CR, complete remission; BMT, allogeneic bone marrow transplantation; As_2O_3, arsenic trioxide; TRM, treatment-related mortality.

maintenance therapy were compared by log-rank test. Overall survival (OS) was calculated from the day of diagnosis to the day of death or censorship. Disease-free survival (DFS) was calculated from the day of diagnosis to the day of confirmed marrow relapse. For salvage cases, analysis of OS was from the day of relapse to the day of death or censorship. The log-rank model was used to analyse differences in OS for the three different treatment methods for relapses (chemotherapy, BMT, As_2O_3-based treatment).

Results

Treatment outcome of newly diagnosed APL

A total of 153 patients were diagnosed with APL within the study period (Figure 1). Complete data for analysis were available in 143 patients. A total of 30 patients died before or during induction chemotherapy. CR1 was achieved in 113 cases. Relapses occurred in 54 patients, at a median of 13 months (range 5–96 months). Late relapses, defined as relapses occurring 2 years after CR1, occurred in 10 cases. The 5-year actuarial DFS from CR1 was 42%. This did not differ significantly for patients with ($n = 59$) and without ($n = 54$) maintenance therapy ($P = 0.087$), owing to the occurrence of more late relapses in the latter group (Figure 2).

Treatment outcome of APL in first relapse

The results of different treatment groups (chemotherapy alone, $n = 19$; chemotherapy followed by allogeneic BMT, $n = 14$; As_2O_3 followed by chemotherapy, $n = 21$) are shown in Table 1 and Figure 3. Ten patients treated with chemotherapy and five patients receiving BMT died from early treatment-related complications. Two patients receiving BMT died from late complications (hepatitis B virus-related liver failure and bronchiolitis obliterans) (Table 2). Durable CR2 was achieved in 30 cases.

Treatment of APL in second or more advanced relapses

There were 11 further relapses at a median of 11 months (range 8–48 months) (Table 1). Both patients in the chemotherapy group received further chemotherapy, and died from treatment-related complications. The only patient who relapsed again in the BMT group received a second allogeneic BMT from an HLA-identical sibling, and has remained in CR3. Two patients in the As_2O_3 group died before further treatment could be given. Six patients achieved and have remained in remission, with combined As_2O_3 plus ATRA therapy.

Statistical analysis

The 2-year actuarial OS from R1 leveled off at 23% for the chemotherapy group, 43% for the BMT group and 82% for the As_2O_3 group (Figure 3). As a result of efficient salvage of advanced relapses, the 5-year actuarial OS from CR1, at 68%, was much better than the DFS.

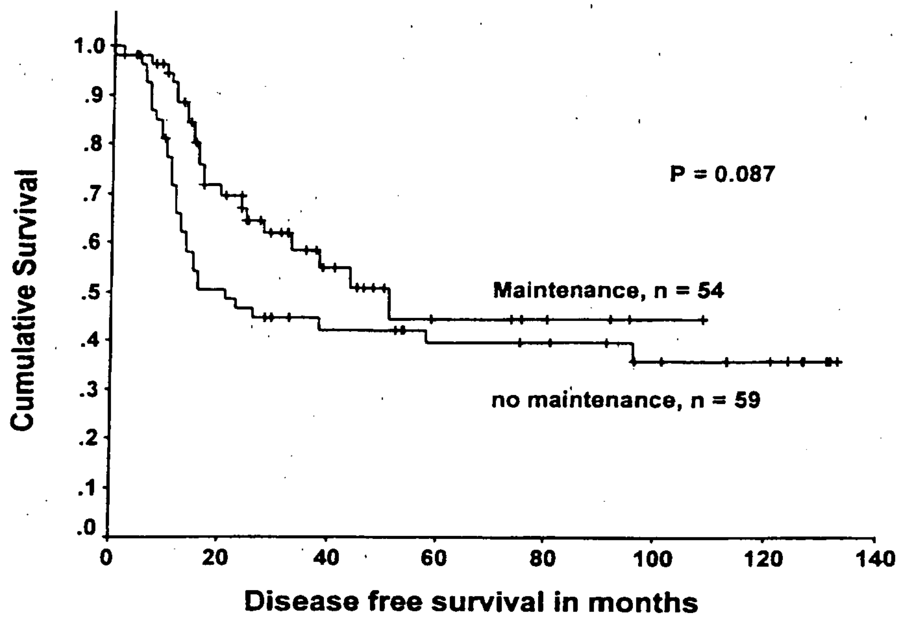

Figure 2. Disease-free survival in 113 acute promyelocytic leukaemia patients with or without maintenance chemotherapy after achieving first complete remission.

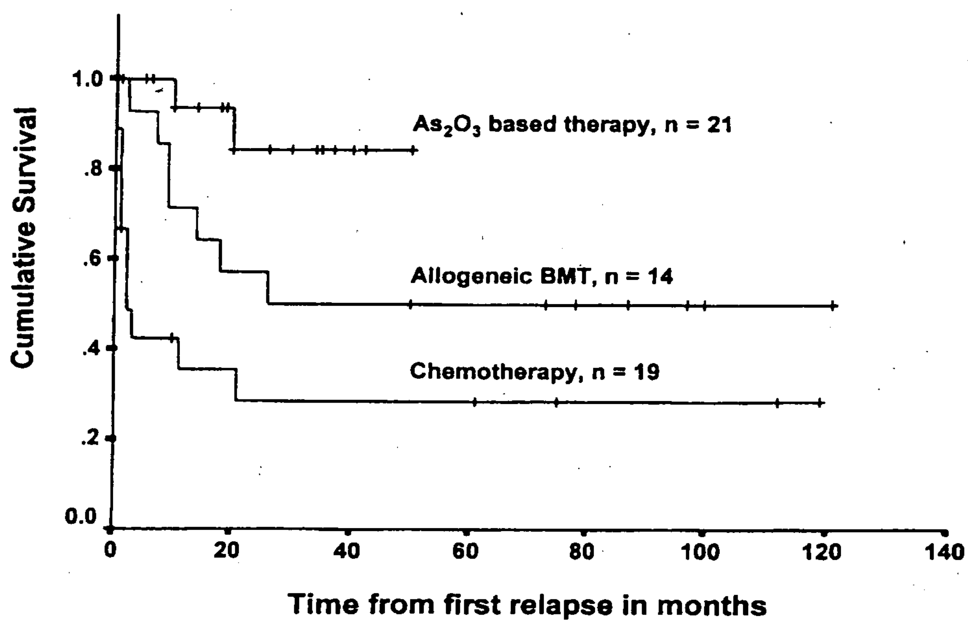

Figure 3. Overall survival of 54 patients with relapsed acute promyelocytic leukaemia treated with arsenic trioxide (As_2O_3)-based therapy, allogeneic bone marrow transplantation (BMT) and chemotherapy.

Discussion

With ATRA and chemotherapy as the induction regimen, the CR rate of 79% observed in this study is comparable to those of 72% and 96% reported previously [9]. However, our 5-year DFS, at 42%, was apparently inferior to the reported 3-year DFS of 86% to 90% in other series [10]. A number of reasons might account for this. One of the four centres in our study has not used maintenance therapy, which has been shown to be of beneficial effect in reducing relapses [11]. Furthermore, chemotherapy tolerance appears to be poor in Chinese people, and full-dose mercaptopurine maintenance was not achievable in most cases [12].

In relapsed APL, allogeneic BMT, chemotherapy and As_2O_3 are all useful treatment modalities, but the best choice and timing of treatment is as yet undefined [13]. Reported data on the use of ATRA plus chemotherapy for relapsed APL showed that only

29–35% of patients could be induced into durable remission [14, 15]. Allogeneic BMT for relapsed patients who achieved a second remission after ATRA plus chemotherapy also gave poor results. In one study, the leukaemia-free survival (LFS) was 22%, relapse rate (RR) was 54%, and the treatment-related mortality (TRM) was 40% [16]. In another study, only two of 11 APL patients in second remission survived the transplantation [17]. Data published by the European Blood and Marrow Transplantation (EBMT) Group showed that in 127 relapsed APL patients who received allogeneic BMT, the LFS was 53–61%, the RR was 10–22% and the TRM was 32–34% [18]. The data from the EBMT appeared to be slightly better than the former two studies, which could be related to different patient selection. However, these studies all showed that allogeneic BMT in APL patients in second remission was associated with a high TRM and an overall unsatisfactory outcome. As for As_2O_3, although a high remission rate can be achieved, the long-term follow-up results are less well defined. In two series comprising 87 relapsed cases, the 18- and 24-month LFS was 56% and 42%, respectively [19, 20].

In comparison with the studies of relapsed APL described above, our data offer a few advantages. This study involved a consecutive series, so that bias related to patient selection for various treatment options was diminished. This was particularly important for BMT, where patient selection could often affect the treatment outcome. Furthermore, the treatment and supportive care were similar. Our results might therefore give a better perspective on the relative merits of the different treatment options in relapsed APL.

We showed that with a follow-up of 3 years, treatment results for BMT were comparable to chemotherapy, but inferior to arsenic-based treatment for relapsed APL. The lower relapse rate with BMT was offset by the high early TRM, a phenomenon also observed in other studies [16–18]. Although few late relapses occurred after BMT, the survival curve remained unstable owing to late deaths from GvHD and organ toxicity. Furthermore, survivors after BMT might still suffer from the permanent side effects of immunosuppression, exposure to alkylating agents, infertility, premature menopause and increased risks of secondary malignancies. In contrast, As_2O_3-based therapy was associated with minimal toxicity or mortality. Although As_2O_3-induced second remissions were apparently associated with more subsequent relapses, long-term remission after combination therapy with As_2O_3 and ATRA might still be achieved in these patients [8]. Furthermore, the lack of organ damage meant that further relapses might still be salvaged by allogeneic BMT, although this was not required in any of our cases. Our results therefore suggest that As_2O_3-based therapy may be the treatment of choice for APL in first or more advanced relapses. For this reason, we have not performed BMT in any APL patients after 1997.

In conclusion, the availability of effective first-line and salvage therapy means that APL patients in any stage of their illness should be treated with curative intent, even when they have late advanced diseases [13]. As_2O_3 appears to be the best option for relapsed cases. The high efficacy of As_2O_3 in inducing second remissions means that optimal consolidation and maintenance of remission are key factors that will improve the cure rate. For allogeneic BMT to be offered as a consolidation, TRM must be improved. On the other hand, APL in advanced and repeated relapse appears to continue to respond to As_2O_3 treatment, which is associated with minimal side effects. Among these options, our data with short-term follow-up seemed to favour the use of repeated courses of As_2O_3. However, prospective clinical trials are needed to fully resolve the issue of As_2O_3 as compared with allogeneic BMT as the optimal treatment for relapsed APL.

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bjh research paper

Phase 2 study of arsenic trioxide in patients with relapsed or refractory multiple myeloma

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Summary

Despite aggressive and innovative therapy, patients with multiple myeloma (MM) invariably relapse and die of their disease. New options for non-cytotoxic salvage therapy and additional therapeutic strategies are needed. Arsenic trioxide, an antitumour agent with a multifaceted mechanism of action, induces apoptosis *in vitro* in MM cell lines and freshly isolated cells from MM patients and, in preliminary studies, displayed clinical activity in patients with late-stage MM. A phase 2, multicentre, open-label study of arsenic trioxide was conducted in 24 MM patients; eight had relapsed and 16 were refractory to prior therapy. Patients received arsenic trioxide 0·25 mg/kg/d for 5 d/week during the first 2 weeks of each 4-week cycle. Sixteen patients had grade 3 or 4 neutropenia and one required antibiotics. Reductions (25% or more) in serum M-protein levels occurred in eight of 24 (33%) patients. An additional six (25%) patients had stable disease. The median time to response was 67·5 d, with a median duration of response of 130 d. Arsenic trioxide therapy lowered serum creatinine levels in two patients with high baseline values. These data indicate that arsenic trioxide is active and reasonably well tolerated as a single-agent salvage therapy, even in patients with late-stage, relapsed and refractory MM.

Keywords: arsenic trioxide, relapsed disease, refractory disease, multiple myeloma, clinical trial.

Multiple myeloma (MM) is characterized by the clonal proliferation of malignant plasma cells in the bone marrow, associated in most patients with the production of monoclonal immunoglobulins. Despite several recent therapeutic advances, MM remains incurable; almost all patients eventually relapse and develop drug resistance. With conventional chemotherapy, the 5-year median survival rate is approximately 25%, and approximately 10% of patients live longer than 10 years (Pandit & Vesole, 2001). High-dose chemotherapy with autologous stem cell transplantation does not improve survival, but it may improve progression-free survival (Pandit & Vesole, 2001; Hussein *et al*, 2002). Additionally, high-dose therapy remains available only to a minority of patients because of its toxicity, which makes it poorly tolerated by patients older than 60 years, the age group primarily affected by MM (Munshi *et al*, 2002). The complex pathophysiology of MM adds to the difficulty of managing the disease successfully. Multiple regulatory pathways involving cytokines, adhesion molecules, angiogenesis and resistance mechanisms control the development and progression of the disease. Recently, successful approaches to non-cytotoxic therapy have been reported with the use of thalidomide alone or in combination with dexamethasone and the proteasome inhibitor, bortezomib (Singhal *et al*, 1999; Richardson *et al*, 2002; Anagnostopoulos *et al*, 2003; Richardson *et al*, 2003). However, although these approaches produce robust responses in a high proportion of patients with late-stage disease, they are of limited duration, and all patients relapse and become refractory to therapy. Additional relatively non-toxic therapeutics that target pathways critical to myeloma cell survival must be explored.

Arsenic trioxide has recently emerged as an effective treatment for patients with relapsed or refractory acute promyelocytic leukaemia (APL). Two US studies have established the safety and efficacy of arsenic trioxide in APL patients for whom intensive chemotherapy failed (Soignet *et al*, 1998, 2001). The overall rate of complete remission was 87%;

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treatment was well tolerated, and adverse events were manageable and reversible. The finding that arsenic trioxide inhibits proliferation and induces apoptosis in a variety of malignant myeloid and lymphoid cells, including myeloma cells that do not contain the promyelocytic-retinoic acid receptor-α (PML-RARα) fusion transcript specific to APL, suggested that it may be active in other diseases (Rousselot *et al*, 1999; Ma *et al*, 2001).

Arsenic trioxide is a promising compound to explore in MM because of its multifaceted antitumour activity. Pharmacologically achievable concentrations of arsenic trioxide affect myeloma cell survival, possibly through the inhibition of glutathione peroxidase, inducing apoptosis and inhibiting the proliferation of MM cell lines and primary MM cells in a dose-dependent manner (Rousselot *et al*, 1999; Park *et al*, 2000; Miller *et al*, 2002). Unlike the antitumour activity of dexamethasone, which is inhibited by interleukin-6 (IL-6), arsenic trioxide-induced apoptosis is not prevented by IL-6 (Rousselot *et al*, 1999; Grad *et al*, 2001). Furthermore, cross-resistance to other chemotherapeutic agents is unlikely with arsenic trioxide because it can induce dose-dependent apoptosis in drug-resistant MM cell lines (Ma *et al*, 2001). Antiangiogenic activities, such as the inhibition of vascular endothelial growth factor production and capillary formation, also promote the antitumour efficacy of arsenic trioxide (Roboz *et al*, 2000; Ma *et al*, 2001). In addition, treatment with arsenic trioxide increases lymphokine-activated killer cell activity and up-regulates CD38 ligand and CD38 on immune effector cells and myeloma cells, indicating that immunomodulation may contribute to its antitumour activity (Deaglio *et al*, 2001; Hussein, 2001).

These experimental studies with MM cells suggest that arsenic trioxide targets multiple mechanisms of disease progression in MM, and they provide the rationale for exploring the clinical efficacy of arsenic trioxide in patients with relapsed or refractory MM. In a pilot study, monotherapy with arsenic trioxide given according to a leukaemia-like schedule produced responses in three of 14 (21%) heavily pretreated patients with advanced MM and prolonged the duration of stable disease in a fourth patient; responses were noted after approximately 2 months of daily therapy and lasted 6 weeks in two patients (Munshi *et al*, 2002). This study showed that arsenic trioxide has acceptable toxicity and promising activity in heavily pretreated patients with MM.

Promising results obtained in the pilot study formed the basis of a phase 2 trial of single-agent arsenic trioxide in patients with relapsed or refractory MM. The objective of the study was to determine the rate of response to a higher, less frequent dosing regimen in patients with MM who had relapsed from or who were refractory to conventional therapies. Because refractory disease constitutes 15–20% of MM cases, the study was designed first to evaluate the response rate in 20 patients with relapsed disease. If the response rate was 20% or greater, the next step was to confirm the response rate in another 35 patients with relapsed disease and to stratify for the type of recurrent disease. Unexpectedly, most patients enrolled in the study had been refractory to previous therapy; thus, we could not test the original hypothesis. Instead, we analysed all patients as one group receiving salvage therapy, and we present a descriptive analysis of the results.

Patients and methods

Study design

This was designed as a multicentre, open-label study of arsenic trioxide in patients with relapsed or primary refractory MM. The institutional review board at each participating institution approved the study and all patients provided informed consent.

Patient selection

Patients with confirmed diagnoses of MM, Durie–Salmon stage II or III, current measurable disease (presence of serum and urine M-protein or measurable plasmacytoma), Karnofsky performance status of at least 70%, and life expectancy of 3 or more months were eligible for the study. Originally, only patients who had undergone three or fewer previous treatment regimens (not more than two cytotoxic regimens and not more than one high-dose cytotoxic regimen as part of stem cell transplantation) and whose most recent therapy was completed at least 28 d before study entry were included. Other criteria for entry were a serum creatinine level of 221 μmol/l or less and serum bilirubin, serum glutamate pyruvate transaminase and serum glutamic-oxaloacetic transaminase levels not greater than 2·5 times the upper limit of normal. However, when it was noted that the drug was well tolerated and that most patients enrolled had been refractory to previous therapy (i.e. patients who progressed within 6 months of previous treatment), these initial entry criteria were broadened to include patients for whom more than three therapeutic regimens had failed.

Patients with reproductive potential used medically acceptable birth control. Supportive care therapy in the form of erythropoietin, bisphosphonates and localized radiation therapy were allowed.

Exclusion criteria included the following: pregnancy or lactation; neurotoxicity or neuropathy of grade 2 or higher; absolute QT interval >460 ms in the presence of serum potassium >4·0 mmol/l and magnesium >0·74 mmol/l; significant cardiac dysfunction including conduction defects, unstable angina, myocardial infarction during the previous 6 months, or New York Heart Association class II or higher congestive heart failure; reduced haematology values (granulocytes $<1{\cdot}2 \times 10^9$/l, platelets $<100 \times 10^9$/l, or haemoglobin <10 g/dl) that were not secondary to MM; any malignancy (other than curatively treated cervical carcinoma *in situ* or non-melanoma skin cancer) in the 5 years preceding the study; uncontrolled diabetes mellitus; active, uncontrolled serious

infections; human immunodeficiency virus infection; history of grand mal seizures; and concurrent treatment with cytotoxic chemotherapy, high-dose corticosteroids (≥20 mg/d prednisone equivalents), broad-field radiation, or investigational agents.

Study treatment

Serum electrolytes were assessed and corrected to potassium greater than 4 mmol/l and magnesium greater than 0·74 mmol/l before the start of treatment and were maintained at these levels throughout the study. Arsenic trioxide (Trisenox; Cell Therapeutics, Inc., Seattle, WA, USA) was administered as an intravenous infusion at a dose of 0·25 mg/kg/d for 5 d/week (Monday to Friday) during the first 2 weeks of each 4-week cycle. The infusion was administered over 1 h unless adverse effects occurred, in which case the infusion was given over 4 h. If arsenic trioxide-related grade 2 or 3 toxicity occurred at the start of the treatment cycle, the cycle was delayed for up to 2 weeks. Standard therapy was administered to treat disease symptoms, infections, or other adverse events, and blood transfusions were allowed.

All patients participated for at least one 4-week cycle of therapy and a 4-week follow-up unless the criteria for unplanned early discontinuation were fulfilled. These included unacceptable toxicity, persistence of treatment-related moderate or severe toxicity for 4 weeks after the end of a treatment cycle and disease progression. Patients who had at least stable disease and who were benefiting clinically continued to receive treatment indefinitely.

Assessment of efficacy

Response to treatment was assessed by quantification of myeloma parameters (serum and urine protein electrophoresis and immunofixation with quantification of the M-protein, Bence–Jones proteins in urine, β_2-microglobulin and C-reactive protein) at screening, every 2 weeks during the treatment period and 4 weeks after the last treatment. An objective response was defined as a 25% or greater reduction in serum M-protein from the prestudy level. Stable disease was defined as less than a 25% decrease or less than a 25% increase in serum M-protein level from baseline that was maintained for at least 8 weeks. A patient was considered to have progressive disease if the M-protein level increased by 25% or more from the lower point of two visits, 2 months apart.

Each patient who completed at least one treatment cycle and who had a baseline measurement and one additional measurement at 8 weeks was evaluated for response.

Assessment of safety

Each patient evaluation included history-taking, medical examination and query regarding adverse events within 4 weeks of the start of arsenic trioxide administration, at each visit during treatment and at the 4-week follow-up. Haematology and clinical chemistry profiles were taken in the week preceding the start of arsenic trioxide treatment, twice weekly during weeks 1 and 2 of each cycle, once during week 4 of each cycle and 4 weeks after the last treatment. An electrocardiogram was obtained at screening, once weekly during weeks 1, 2 and 4 of each cycle, and at follow-up. Patients who received at least one dose of arsenic trioxide were assessed for safety.

Results

Patients' characteristics

Of the 25 patients enrolled in the study: one patient withdrew consent before the start of treatment and 24 received the study drug. Baseline demographic and clinical characteristics of these patients are shown in Table I. Patients ranged in age from 41 to 80 years, with a median age of 63 years; 13 of the 24 (54·2%) patients were men. Twenty (83·3%) patients had

Table I. Patient characteristics.

Number of patients	24
Sex, *n* (%)	
Men	13 (54·2)
Women	11 (45·8)
Age, years, median (range)	63 (41–80)
≥60 years, *n* (%)	14 (58·3)
<60 years, *n* (%)	10 (41·7)
Performance status, *n* (%)	
100	2 (8·3)
90	6 (25)
80	11 (45·8)
70	5 (20·8)
Durie–Salmon stage, *n* (%)	
II	4 (16·7)
III	20 (83·3)
Disease status, *n* (%)*	
Relapsed	8 (33·3)
Refractory	16 (66·7)
Previous chemotherapy regimens, *n* (%)	
1	1 (4·2)
2	11 (45·8)
3	6 (25)
>3	6 (25)
Previous autologous stem cell transplantation, *n* (%)	7 (29·2)
Time since diagnosis, years, median (range)†	2·4 (0·4–6·0)
β2-microglobulin (mg/dl), median (range)	4·0 (1·9–16·1)
Platelets ($\times 10^9$/l), median (range)‡	171 (2–382)
Haemoglobin (g/dl), median (range)‡	10·3 (5·7–14·8)

*Refractory status refers to patients who received arsenic trioxide within 6 months of prior treatment; relapsed status refers to patients who received arsenic trioxide more than 6 months after prior treatment.
†Based on 18 patients.
‡Based on 23 patients.

stage III disease, and 16 (66·7%) patients had been refractory to previous treatment. All refractory patients had relapses within 3·5 months of receiving their last treatment. Patients were heavily pretreated; all but one of the patients had received two or more previous chemotherapy regimens (six of these patients received more than three regimens), seven patients received thalidomide (alone or in combination with glucocorticoids), and seven patients underwent autologous stem cell transplantation. Eight (33·3%) patients had haemoglobin levels <10 g/dl, and nine (37·5%) patients had platelet counts $<150 \times 10^9$/l.

Patient disposition

Of the 24 patients, 22 completed at least one cycle of arsenic trioxide therapy. One of the two patients who did not complete one cycle withdrew consent; treatment was discontinued in the second patient because of grade 3 anaemia (unrelated to arsenic trioxide therapy) and grade 3 rectal haemorrhage that was possibly related to arsenic trioxide therapy. Sixteen patients received two or more cycles of therapy: two cycles ($n = 6$), three cycles ($n = 2$), four cycles ($n = 2$), five cycles ($n = 2$), six cycles ($n = 3$) and more than six cycles ($n = 1$).

Disease response

Reponses to treatment, as defined by reductions in paraprotein or Bence–Jones protein levels, are summarized in Table II. Objective response rates of 25% or greater reduction in serum M-protein occurred in eight of the 24 (33·3%) patients. Stable disease (<25% reduction or <25% increase in serum M-protein level from baseline) was noted in an additional six of the 24 (25%) patients. Five (20·8%) patients had progressive disease and the remaining five patients could not be assessed for a response (two patients did not complete one cycle of therapy; one patient was removed from the study because of increasing leg pain, began radiation and dexamethasone therapy, and refused follow-up tests; and two patients had stable disease through cycle 1, but no confirmatory assessment was made at 8 weeks).

Individual treatment responses for the patients who had objective responses or whose disease was stabilized after arsenic trioxide therapy are shown in Table III. The time to paraprotein level reduction or stabilization ranged from 12 to 263 d, with a median of 67·5 d. Median duration of response was 130 d (range 25–515+ d).

Table II. Treatment responses.

Response criteria	No. of patients (%)
Objective response	8 (33·3)
Stable disease	6 (25)
Progressive disease	5 (20·8)
Not evaluable	5 (20·8)

Safety assessment

Adverse events related to arsenic trioxide therapy and experienced by more than 10% of patients are listed in Table IV. Most adverse events were mild to moderate (grades 1 and 2), and non-haematological adverse events of greater severity were rare. Grades 3 and 4 neutropenia occurred in 11 (45·8%) and five (20·8%) of the 24 patients, respectively. However, only one patient with grade 4 neutropenia required growth factor support and systemic antibiotic treatment for a bacterial infection. Febrile neutropenia (grade 3) was reported in only one patient.

Renal function did not deteriorate during arsenic trioxide therapy in any of the patients; rather, creatinine levels returned to normal during arsenic trioxide treatment in two patients with high baseline levels of serum creatinine (194·5 and 168 μmol/l respectively).

Discussion

This phase 2 study showed that arsenic trioxide as monotherapy has therapeutic efficacy in relapsed or refractory MM. The study was originally designed to assess response rates in patients with relapsed disease and to qualitatively analyse data from the expected smaller number of patients with refractory disease. Most patients in the study were refractory to previous therapeutic regimens, including seven who were also refractory to thalidomide. Traditionally, refractory patients are difficult to manage because they are unlikely to respond to further therapeutic manipulations (Pandit & Vesole, 2001). Arsenic trioxide single-agent therapy resulted in a clinical benefit in this poor-prognosis group. Objective responses were obtained in 33% of patients, and an additional 25% attained stable disease, contributing to an overall benefit in 58% of patients on an intent-to-treat basis. Responses (objective responses and stable disease) to arsenic trioxide were gradual and occurred within a median of 67·5 d of treatment. In particular, objective responses occurred after at least three cycles of therapy, and in one patient (patient 7), an objective response occurred after 10 cycles. Disease response and stabilization were maintained for a median of 130 d (range 25–515+ d). One patient received 19 cycles of therapy and continues on therapy.

Therapy with arsenic trioxide was well tolerated with manageable adverse events. As expected in this heavily pretreated population, 67% of the patients experienced grade 3 or 4 neutropenia. However, this neutropenia was not associated with an increased incidence of neutropenic fevers or an increased use of growth factors. Only one of the 16 patients with grade 3 or 4 neutropenia required antibiotic and growth factor support for infection. QT prolongation, a known side effect of arsenic trioxide therapy (Soignet *et al*, 2001; Barbey *et al*, 2003), was uncommon in this study and, when noted, did

Table III. Individual treatment responses.

Patient	Age (years)/ sex	No. of prior chemotherapy regimens	Disease status	Time since last treatment (months)	No. of arsenic trioxide cycles	Best response achieved	Change in serum M-protein level from baseline (%)	No. of days to best response	Duration of response (days)
2	79/M	2	Rel	36·99	5	OR	−39	68	165
3	52/M	2	Ref	1·81	6	OR	−32	152	193
4	57/F	2	Ref	2·4	5·5	OR	−25	96	163+
5	73/M	2	Rel	5·19	4	SD	+2	68	138+
6	66/M	3	Ref	1·84	6	SD	+22	40	201+
7	73/F	5	Ref	1·81	19	OR	−38	263	515+
9	46/F	2	Ref	1·84	2	SD	−13	12	66
10	80/M	2	Ref	2·46	2	SD	−10	12	51
12*	57/M	3	Ref	1·51	1·5	OR	−41	12	25+
14	66/F	3	Ref	1·08	2	SD	−10	12	71
18	76/M	2	Rel	57·07	6	OR	−25	134	176
19	77/F	1	Rel	7·46	4	OR	−34	92	122
21	60/F	2	Ref	1·22	3	OR	−33	67	81
22	56/M	5	Ref	2·3	2	SD	+19	12	53+

Rel, relapsed; Ref, refractory; M, male; F, female; OR, objective response; SD, stable disease.
*Patient died of glioblastoma.

Table IV. Treatment-related adverse events.

Event	Number of patients (%)		
	Grade 3	Grade 4	Total*
Haematological toxicities			
Neutropenia	11	5	17 (71)
Anaemia NOS	5	0	9 (38)
Thrombocytopenia	5	0	7 (29)
Non-haematological toxicities			
Nausea/vomiting	1	0	19 (79)
Fatigue/asthenia	2	0	18 (75)
Dyspnoea	2	0	14 (58)
Abdominal pain	1	0	8 (33)
Infection (herpes zoster/ herpes simplex)	1	0	8 (33)
Liver function profiles increased	1	0	6 (25)
Pyrexia	1	0	5 (21)

NOS, not otherwise specified.
Grades are in accordance with maximum National Cancer Institute common toxicity grades (version 2).
*Total reflects all grades.

not require any specific intervention. The frequency of grade 3 or 4 non-haematological adverse events was also low. Renal function did not deteriorate during therapy, and treatment with arsenic trioxide normalized creatinine levels in two patients whose creatinine levels were higher than 132·6 μmol/l at the start of therapy.

Our findings highlight some important features in the clinical management of MM with arsenic trioxide when compared with chemotherapy. The increased incidence of neutropenia that was not associated with neutropenic fevers and not requiring intervention in the form of suspended therapy, reduced dose, growth factor use, or antibiotic therapy, is remarkable. Renal function was not compromised by arsenic trioxide therapy and actually improved during treatment in two patients with initially high serum creatinine levels. Overall, arsenic trioxide therapy did not compromise the patients' performance status and did not confer significant added toxicity.

Objective responses and disease stabilization resulting from arsenic trioxide therapy in this study were gradual and consisted of modest, but durable, reductions in paraprotein levels after an average of three cycles of therapy. Similarly, in MM patients who received thalidomide, reductions in paraprotein levels were apparent within approximately 2 months (Singhal *et al*, 1999).

Disease stabilization in the management of MM (i.e. non-responsive, non-progressive patients) appears to provide patients with the same duration of progression-free survival as responders (Browman *et al*, 1995). The clinical significance of response to therapy, as measured by the paraprotein level, has been controversial because the high incidence of complete and near complete remissions after bone marrow transplantation has not led to improvements in survival (Blade *et al*, 1998). In addition, the degree of initial response does not significantly influence survival because survival rates in patients who achieve complete remission have been found to be similar to those of patients with objective or partial responses (Blade *et al*, 1998). More recently, the concept of a plateau phase has been introduced for evaluating the response in MM. The plateau phase is a cytokinetically stable period after chemotherapy for MM and is characterized by stabilization of M-protein levels without further tumour regression, despite continued therapy (Durie *et al*, 1980). An 8-year follow-up study of 432 MM patients demonstrated that a

plateau phase lasting more than 12 months was a stronger predictor of survival after conventional chemotherapy than was the degree of response, measured as the percentage reduction in paraprotein level (Oivanen, 1996). A recent analysis of South West Oncology Group trials of standard-dose chemotherapy in patients with newly diagnosed MM showed that survival is influenced more by the occurrence of progression than by the presence of a response and that time to first progression is the best indicator of survival (Durie *et al*, 2002). Survival rates were similar in non-responders with non-progressive disease and in responders who had ≥50% or ≥75% reductions in paraprotein levels, thus confirming earlier findings (Browman *et al*, 1995).

As arsenic trioxide influences the bone marrow microenvironment in MM through immunomodulatory and antiangiogenic actions, a gradual antitumour effect should be anticipated. Response rates achieved with arsenic trioxide are similar to those obtained in MM with other non-traditional agents, such as thalidomide, that have strong immunomodulatory activities (Singhal *et al*, 1999). In the absence of progressive disease and adverse events, arsenic trioxide treatment should be continued because it maintains disease stability and could possibly prevent renal deterioration. Improvements in severely impaired renal function in patients with late-stage MM using arsenic trioxide in combination with ascorbic acid and low-dose melphalan have been recently described (Borad *et al*, 2003).

The clinical efficacy and favourable toxicity profile of arsenic trioxide demonstrated in this study argue for the further evaluation of arsenic trioxide in relapsed or refractory MM using new dosing strategies (e.g. a loading dose with a schedule requiring less frequent maintenance) combined with traditional chemotherapeutic agents, such as melphalan and dexamethasone, or non-traditional agents, such as bortezomib and thalidomide. In particular, the finding that arsenic trioxide at non-cytotoxic concentrations increases the sensitivity of MM cells to killing by melphalan, dexamethasone, or bortezomib suggests the potential for combination strategies that provide antitumour efficacy with reduced toxicity (Ma *et al*, 2001).

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Expert Opinion

Arsenic trioxide for the treatment of myelodysplastic syndromes

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The impressive activity of arsenic trioxide in acute promyelocytic leukaemia (APL) has renewed the interest in this old compound. Arsenic trioxide targets the sulfhydryl groups present in many proteins involved in oncogenesis and has a broad spectrum of biological activities. This article will review the mechanisms of action of the drug and their relevance to the treatment of myelodysplastic syndrome (MDS), a disease for which no standard treatment currently exists. The early clinical experience has confirmed the activity of arsenic trioxide in MDS. The preliminary results of ongoing Phase II studies conducted in patients with MDS suggest that arsenic trioxide produces haematological improvement including durable transfusion independence in ~ 30% of patients. The current data are presented and discussed in this review.

Keywords: arsenic trioxide, chemotherapy, myelodysplastic syndromes

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1. Introduction

Arsenic has been used as therapeutic agent and a poison for 2400 years [1]. The effects of arsenic on the haematopoietic system have been known since the nineteenth century. A solution of arsenic trioxide developed by Fowler was shown to induce a dramatic reduction of white blood cell counts in patients with leukaemia while having a small impact in normal individuals [2]. Until the beginning of the twentieth century, Fowler's solution was the mainstay of leukaemia treatment and particularly of chronic myelogenous leukaemia (CML). With the developments of therapeutic irradiation and chemotherapy, the use of arsenic declined in the mid-twentieth century. Reports of chronic poisoning in patients treated for CML and the discovery of carcinogenic potential of the arsenic derivatives also contributed to this decline. Studies conducted in the 1960s showed that the activity of arsenic in the treatment of malignancies related to its capacity to bind to the sulfhydryl groups present in many proteins involved in oncogenic pathways. However, the renewed use of arsenic is due to the discovery of its remarkable activity in acute promyelocytic leukaemia (APL) in the 1970s. Investigators in Harbin, China reported that the 'Ai Lin-1', a solution containing arsenic trioxide, was able to induce complete remission in patients with APL. Their results were confirmed by clinical and biological studies conducted in Shangai [3] and then at the Memorial Sloan-Kettering Cancer Center [4]. In a large multicentre, Phase II study, the drug was shown to produce complete remission in 85% of patients with relapsed APL including patients achieving molecular remission [5]. The impressive activity of the drug in APL led to its approval in the US and in Europe under the brand name of Trisenox® (arsenic trioxide) for 'the induction of remission and consolidation in patients with APL whose conditions are refractory to or who have relapsed from retinoid and anthracycline chemotherapy and whose APL is characterised by the presence of the t(15;17) translocation or promyelocytic leukemia-all-trans-retinoic acid (PML-RARα) gene expression' [6].

This discovery also prompted extensive biological investigation which has revealed the wide range of biological activities of arsenic trioxide. The drug is able to induce differentiation and apoptosis and to inhibit cell proliferation or angiogenesis

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Table 1a. International Prognostic Scoring System.

	Score value				
Prognostic variable	0	0.5	1.0	1.5	2.0
Marrow blasts (%)	< 5	5 – 10	NA	11 – 20	21 – 30
Karyotype	Good	Intermediate	NA	Poor	NA
Cytopenias	0 or 1	2 or 3	NA	NA	NA

Karyotypes: Good = Normal or any one of:-Y, del(5q) or del(20q); Poor = -7 or other chromosome 7 anomalies or complex: more than three abnormalities; Intermediate = all other abnormalities. The cytopenias are defined by neutrophil count < 1800/µl, platelets < 100,000/µl or haemoglobin < 10 g/dl.
NA: Not available.

Table 1b. Survival by risk category.

Risk category	Combined score*	Median survival
Low	0	60 months
Intermediate-1	0.5 – 1.0	36 months
Intermediate-2	1.5 – 2.0	18 months
High	≥ 2.5	4 months

*Combined score = Sum of marrow blast + karyotype + cytopenia scores. Adapted from [11].

and therefore, has the potential to be active in tumour models other than APL [7] among which, myelodysplastic syndromes (MDS) are the most promising.

2. Clinical features of myelodysplastic syndromes

MDS represent a heterogeneous group of clonal diseases of the haematopoietic stem cells. The hallmark of the disease is ineffective haematopoiesis, characterised by marrow dysplasia with incomplete maturation and progressive increase in the percentage of myeloblasts. The incidence of MDS has been reported to be 4 – 5/year/100,000 [8] but is probably underestimated as many cases may not be diagnosed. The median age is ~ 65 years and the incidence of the disease increases with age. With the ageing of the general population in developed countries, the incidence of MDS is expected to increase in the near future.

Most of the clinical symptoms are related to neutropenia, anaemia or thrombopenia, which are present at various degrees and tend to deteriorate with time. They include infections, fatigue, dyspnoea and bleeding. The majority of patients will require red blood cells (RBC) or platelet transfusions during the course of their disease. Approximately 20 – 40% of cases will transform into acute myelogenous leukaemia (AML) after various lengths of time, whilst the remaining patients die from infection, bleeding or complications of repeated RBC transfusions.

For many years, MDS were regarded as complex diseases with heterogeneous clinical presentations, evolution and survival. The French–American–British (FAB) classification was the first to correlate prognostic with cyto-morphological features and has been widely used [9]. It recognised five different forms according to the blast percentage: refractory anaemia (RA), refractory anaemia with ringed sideroblasts (RARS), refractory anaemia with blast excess (RAEB), refractory anaemia with blast excess in transformation (RAEB-t) and chronic myelo-monocytic leukaemia (CMML). This classification illustrates the continuum between MDS and AML, which share many common biological features. Increasing blast counts correlate with the risk of AML transformation and with a short survival. However, other important independent prognostic variables such as cytogenetics, are not recognised by this classification [10]. Recently, the International Prognosis Scoring System (IPSS) has improved the prognostic classification of patients with MDS [11]. A simple and reproducible score allows for the stratification of patients into four groups with 60, 36, 18 and 4 months, respectively, median survival, on the basis of three parameters: the number of cytopenia, cytogenetic abnormalities and the percentage of marrow blasts (Tables 1a and 1b). In addition to this prognostic scoring system, the definition of standardised criteria for response evaluation by the International Working Group (IWG) that are presented in Table 2, represent major advances for the clinical research in MDS [12].

3. Available therapeutic options

There is currently no standard treatment for MDS and supportive care is still the mainstay of MDS treatment. RBC or platelet transfusions are given to treat fatigue or bleeding symptoms together with antibiotics when infections occur. Treatment with erythropoetin alone or in combination with granulocyte colony stimulating factor (G-CSF) can improve anaemia and provide transfusion independence in ~ 30 – 40% of patients [13,14]. The benefit of treatments with growth factors is clearly restricted to the patients who have low marrow blast

Table 2. International Working Group (IWG) standardised response criteria for myelodysplastic syndrome.

Altering disease natural history	
Complete remission	< 5% marrow blast, no evidence of dysplasia
Partial remission	Absolute neutrophil count > 1500/mm³ and haemoglobin > 11 g/dl and platelets > 100,000/mm³ All complete remission criteria except marrow blast > 5% with a 50% decrease from baseline
Haematological improvement	
Erythroid response	**Major**: transfusion independence or > 2 g/dl increase in haemoglobin **Minor**: 50% decrease in transfusion requirements or 1 – 2 g/dl increase in haemoglobin
Platelet response	**Major**: absolute increase of ≥ 30,000/mm³ or transfusion-independence **Minor**: increase of ≥ 50% in platelet counts with a net increase of > 10,000/mm³ but < 30,000/mm³
Neutrophil response	**Major**: 100% increase or absolute increase of > 500/mm³ **Minor**: 100% increase but absolute increase of < 500/mm³
Cytogenetic response	
	Major: no detectable cytogenetic abnormality, if pre-existing abnormality was present **Minor**: ≥ 50% reduction in abnormal metaphases
Quality of life assessment	
	Measured by instruments such as the FACT questionnaire

Adapted from [12].
FACT: Functional Assessment of Cancer Therapy.

percentage, low endogenous erythropoetin levels (< 500 UI/l) and low RBC requirements (< 2 U/month) [15]. In the favourable group, the response rate is 60% and responses were shown to correlate with quality of life improvements [15].

Allogeneic stem cell transplantation (SCT) is the only curative approach but its use is limited by the need for an human leukocyte antigen (HLA) identical donor and by the toxicity of the procedure which precludes its use in old patients [16,17]. For these reasons, the use of allogeneic SCT has been restricted to a minority of young patients. However, with the recent developments of non-myeloablative conditioning regimens, allogeneic SCT can be offered to patients up to 70 years of age and becomes an important option for MDS.

Various cytotoxic therapies have been investigated over the past 20 years but none have been shown to improve survival. They include low-dose cytosine arabinoside (5 – 20 mg/m² daily by subcutaneous infusion 2 weeks each month) which produces response in ~ 30% of patients ranging from haematological improvements (HI) (correction of cytopenias) to complete remission [18,19]. However, the duration of responses is usually brief and in a substantial proportion of patients, the treatment is associated with a worsening of cytopenias and to significant mortality [18]. Recently, several studies have investigated the use of intensive chemotherapy regimen similar to those used for the treatment of AML. With the combination of anthracycline and intermediate- or high-dose cytarabine, complete remission rates are ~ 50 – 60% but the response duration and survival are short, rarely exceeding a median of 1 year [10,20,21]. The introduction of new chemotherapeutic agents such as the topoisomerase I inhibitor topotecan has not significantly improved the results of intensive treatments but the drug represents an interesting alternative to anthracyclins and may reduce the induction mortality in elderly patients [22,23]. Other attempts to improve these results have included the use of autologous SCT which was shown to provide long-term disease free survival in a subset of patients [24,25]. However, when formally compared to conventional chemotherapy, there was no clear advantage supporting the use of autologous SCT in MDS [26].

With the increase in our understanding of the biology of MDS [27], several therapeutic alternatives to chemotherapy have been investigated. These approaches are directed to the abnormal pathways found in the myelodysplastic clone, such as differentiation, apoptosis or angiogenesis (Table 3). Impaired differentiation is a biological hallmark of MDS. Treatment with the differentiating agents – cis-retinoic acid or all-trans retinoic acid (ATRA) – have been shown to induce responses in patients with low-risk MDS [28-30]. However, the response rate were limited (complete remissions and HI rates of 4 and 12%, respectively) without improvement of survival [28,31].

Thalidomide recently emerged as a major antiangiogenic agent with impressive clinical activity in various tumour models and especially in myeloma. Increased angiogenesis reflected by high microvessel density and overexpression of VEGF has been documented in MDS [32-34] and support the rationale for the investigation of thalidomide. Raza *et al.* [35] reported the results of a Phase II study of thalidomide in 83 patients with MDS. Response rate was 20% by intent-to-treat and 31% in evaluable patients, including patients who achieved transfusion-independence. Although escalating doses of thalidomide were used, 38% of patients could not tolerate treatment and discontinued within the first 12 weeks. These results have confirmed that angiogenesis is an important therapeutic target in MDS and underline the need for new and less toxic compounds, such as the vascular endothelial growth factor

Table 3. Principal pathways involved in myelodysplastic syndrome pathogenesis.

Pathway	Molecular alterations	Cellular consequences
Apoptosis	Increased TNF-α Increased Fas-L	Ineffective haematopoiesis Note that the apoptotic fraction decreases with MDS evolution
Angiogenesis	Increased VEGF	Tropic signals to myeloid cells Proliferation
Genomic instability	Defective DNA MMR	Accumulation of somatic events Clonal evolution
Hypermethylation	p15NK4B silencing	Cell cycle alterations Clonal evolution

MDS: Myelodysplastic syndrome; MMR: Mismatch repair; VEGF; Vascular endothelial growth factor.

(VEGF) inhibitors SU5416 and CC5013, which are currently under investigation [36,37].

Hypomethylating agents represent another therapeutic class with promising activity in MDS. Decitabine [38,39] and azacytidine [40] were shown to induce differentiation of malignant cells *in vitro* by hypomethylation of DNA. The overall response rate (including complete remission, partial remission and HI) following treatment of various stages MDS with decitabine is close to 50% [41] including cytogenetic responses in 30% of patients [39]. Interestingly, the response rate seemed higher in patients with a high-risk IPSS score [41]. More recently, Silverman *et al.* [40] reported the results of a prospective, Phase III, controlled study comparing azacytidine to best supportive care in 191 patients with MDS. Results show that azacytidine induces responses in 60% of patients compared to 5% in the controlled arm. Patients in the azacytidine group also had prolonged time to AML transformation and survival when compared to supportive care. In addition, these effects were associated to a significant improvements of quality of life [42].

4. Arsenic trioxide

4.1 Mechanisms of action

The activity of arsenic in APL is mediated by the induction of differentiation and apoptosis. Arsenic induces the degradation of the PML and PML-RARα proteins both *in vitro* and *in vivo*, which allows the APL cells to overcomes the differentiation block which is the hallmark of the disease [43]. The induction of apoptosis on the other hand is independent from PML-RARα and, therefore, may be achieved in other cancers [44]. In addition to the induction of differentiation and apoptosis, arsenic trioxide has been shown to inhibit cell growth, proliferation and angiogenesis [7]. Arsenic trioxide has pleiomorphic activities which support the rationale for its use in several cancer models and particularly in MDS.

Oxidative damage is the main mechanism by which arsenic induces apoptosis. The exposure of cells to arsenic is associated to the production of reactive oxygen species (ROS), which is followed by the accumulation of hydrogen peroxide due to a block of glutathione transferase and glutathione peroxydase metabolic activities [45]. This leads to inner mitochondrial membrane potential disruption, cytochrome-C release and activation of an apoptotic caspase cascade [46]. Glutathione depletion increases the biological effects of arsenic [47,48]. This can be achieved *in vivo* with ascorbic acid, which has synergistic effects with arsenic trioxide [49,50]. Alternative mechanisms of apoptosis induction have been described following exposure to arsenic including disruption of the mitotic process by inhibition of microtubule assembly [51], downregulation of the antiapoptotic protein, bcl-2 [52,53], and inhibition of NF-κB activity [53].

Arsenic can inhibit angiogenesis through the induction of apoptosis in endothelial cells [54] but also by a direct inhibition of VEGF production [55]. Finally, arsenic was shown to have antiproliferative activity due to blocking of the G_2/M-phase of the cell cycle[56] or inhibition cyclin-dependent kinases [57].

4.2 Chemistry

Arsenic trioxide (Trisenox®, Cell Therapeutics, Inc.) is a trivalent inorganic arsenical. The molecular formula of the drug substance in the solid-state is As_2O_3, with a molecular weight of 197.8 Da. Arsenic trioxide is available in 10 ml ampoules for intravenous infusion containing arsenic trioxide 10 mg.

4.3 Pharmacokinetics and metabolism

Pharmacokinetic data of arsenic trioxide are still limited because the active metabolites are not clearly defined and because of an extensive accumulation of the drug into tissues, which makes pharmacokinetic studies difficult.

After intravenous infusion or oral ingestion, arsenic trioxide rapidly distributes to liver, kidneys and spleen after acute or chronic exposure. In pregnant animals, arsenic trioxide has been shown to cross the placenta barrier when given orally or by injection with a distribution in the late-term fetus similar to adult animals. The trivalent form of arsenic is metabolised by methylation to methylarsonic acid (MAA) and to dimethylarsinic acid (DMMA). The reaction requires functional methyltransferases and takes place in the liver although the blood and kidney also participate. During chronic administration, the quantity of DMAA excreted in the urine increases whereas other metabolites decrease. The major pathway of elimination of arsenic compounds in animals is renal excretion. Faecal excretion accounts for < 10% of the total arsenic. Other route of elimination include lung, sweat, milk, hair and skin. The

early half-life is 2 – 6 h with a slower phase taking place with a half-life of 1 – 15 days during which DMAA is the prevalent excreted form. Arsenic concentration measured in hair and nails gradually increased over a 60-day treatment regimen with levels five to seven times higher than baseline levels [3]. In patients with various malignancies, pharmacokinetic studies of total arsenic have shown maximum conentration (C_{max}) values of 0.3 – 2.9 µM in plasma and 0.45 – 2.4 µM in RBCs with a large volume of distribution at steady-state (400 l) consistent with extensive tissue or plasma binding [58].

4.4 Pharmacodynamics

The majority of pharmacodynamic studies of arsenic trioxide have been conducted in patients treated for APL. Arsenic trioxide was shown to induce partial differentiation of APL cells with the accumulation of atypical myeloid cells co-expressing early (CD33) and late (CD11b) differentiation antigens in the bone marrow [4]. Caspase induction and activation were also measured in APL patients after treatment with arsenic trioxide [4].

In patients treated with arsenic trioxide for MDS, we have studied the induction of apoptosis using measurements of annexin V expression in bone marrow cells. Analyse were performed on the bone marrow mononucleated cells and on the CD34-positive fraction at 8 and 16 weeks of treatment. A significant increase of annexin V expression in the nine patients analysed at week 8 compared to baseline was observed [Mohty & Vey, unpublished data].

4.5 Safety and tolerability

The most common adverse events (those occurring in > 25% of patients) recorded in patients with relapsed APL or advanced haematological malignancies treated with 0.15 mg/kg/day up to 60 days were nausea, vomiting, diarrhoea, abdominal pain, sore throat, fatigue, pyrexia, oedema, rigors, cough, dyspnoea, headache, insomnia, hyperglycaemia, hypokalaemia, dermatitis, pruritus and tachycardia.

Arsenic trioxide can cause QT prolongation and complete atrio-ventricular block [59,60]. QT prolongation can lead to torsades de pointes. However, with a strict monitoring of potassium and magnesium serum levels, of electrocardiogram and of the use of other drugs that prolong QT, no serious cardiac adverse events were observed in the postmarketing experience with arsenic trioxide.

A differentiation syndrome reminiscent of the retinoic acid syndrome has been described in patients treated with arsenic trioxide for relapsed APL [61]. Hyperleucocytosis has been recorded in APL patients and seen exclusively during induction treatment. Among the serious adverse events, peripheral neuropathy with a stocking-glove distribution of dysesthesia has been reported during arsenic trioxide treatment. Symptoms were mild-to-moderate in most cases and reversible upon cessation of treatment.

Some degree of myelosuppression has been observed in the studies of arsenic trioxide in patients with MDS and myeloma

Table 4. Preliminary results of the European Phase II study of arsenic trioxide in patients with myelodysplastic syndrome.

	Lower-risk group	Higher-risk group	Total
Evaluable patients	16	21	37
Complete/partial responses	0	0	0
Haematological improvement	3 (19%)	6 (29%)	9 (24%)
Stable disease	NA	11 (52%)	NA
Progressed to AML	1 (6%)	4 (19%)	5 (13%)

AML: Acute myelogenous leukaemia; NA: Not applicable.

(Phase II of arsenic trioxide single agent in MDS), 26 of the 51 (50%) evaluable patients presented Grade 3 – 4 neutropenia (grade based on percentage change from baseline according to the National Cancer Institute–Common Toxicity Criteria (NCI/CTC) Version 2.0) and 16 (31%) patients presented Grade 3 – 4 thrombopenia. Myelosuppression was transient and resolved without drug discontinuation.

A postmarketing survey including 2600 patients was recently reported [63]. It confirmed the absence of serious cardiac side effects and showed that the adverse-effects of arsenic trioxide are manageable and reversible. Arsenic trioxide is well-tolerated and has a non-chemotherapy-like toxicity profile. It does not require the use of central catheters and can be administered on an out-patient basis. These are important considerations for the MDS population which includes a large proportion of elderly patients.

4.6 Clinical efficacy

The clinical efficacy of arsenic trioxide for the treatment of MDS is currently under investigation [27]. Two single-agent Phase II studies are being conducted in the US (CTI-1058 protocol) and in Europe (CTI-1061 protocol). In both studies, patients with various FAB subtypes and all IPSS categories are eligible. Disease evaluation are performed every 8 weeks and the patients are scheduled to receive a minimum of 16 weeks of treatment. Different treatment schedules are used in the two studies. In the European study, patients are scheduled to receive a loading dose of arsenic trioxide 0.30 mg/kg/day as a 1-h intravenous injection for 5 consecutive days followed by a twice-weekly infusion of 0.25 mg/kg for 15 weeks. A preliminary analysis was recently performed on the first 45 patients for whom data were available [62]. The median age was 67 years (range: 42 – 81 years). All FAB categories were represented: 6 patients have RA, 6 patients RARS, 25 patients RAEB, 6 patients RAEB-t and 2 patients CMML. A total of 38% were in the lower risk group (low- and intermediate-1 risk categories of the IPSS) and 62% in the higher risk group (intermediate-2 and high risk categories). A total of 37 (82%) patients were transfusion-dependent. Of the

Table 5. Characteristics of the nine responders in the European Phase II study of arsenic trioxide.

UPN	Age (years)	FAB	Karyotype	Treatment duration (weeks)	Response (time to progression)
Low-risk group					
61008	67	RA	Diploid	24	Major HI-E (43+)
61022	53	RAEB	Diploid	16	Major HI-E/major HI-P (17+)
61034	60	RARS	Diploid	9	Major HI-E (13+)
High-risk group					
61009	76	RAEB	Diploid	169	Major HI-P (17+)
61015	68	RAEB-t	Diploid	8	Major HI-P (34+)
61024	72	RAEB	Complex	16	Major HI-E (12)
61013	81	RAEB	Complex	8	Major HI-E (17+)
61027	70	RAEB	Trisomy 8	24	Major HI-N (30+)
61030	70	RAEB	Complex	24	Major HI-E/major HI-N (17+)

FAB: French–Bristish–American; HI-E: Haematological improvement erythroid response; HI-P: Haematological improvement platelet response; HI-N: Haematological improvement neutrophil response; RA: Refactory anaemia; RAEB: Refactory anaemia with blast excess; RAEB-t: Refactory anaemia with blast excess in transformation; RAR: Refactory anaemia with ringed sideroblasts; UPN: Unique patient number.

patients who had received at least 8 weeks of treatment, 30 could be evaluated for response. As shown in Table 4, 9 (24%) patients achieved an HI and 11 (52%) patients in the higher risk group had stable disease. Responses were seen across the three haematological lineages and five patients became transfusion independent (Table 5). No partial or complete remission have been recorded in this group of patients.

In the US study, arsenic trioxide 0.25 mg/kg/day as an intravenous injection, 5 days/week for 2 weeks, followed by 2 weeks with no therapy (2 weeks on/2 weeks off). In a preliminary report of 30 evaluable patients [64], 1 patient with CMML had achieved a complete remission and 9 patients a HI with 3 patients achieving transfusion-independence. The overall response rate in this study is 30%.

Another study conducted at the Rush University, Chicago by Raza *et al.* [35] is currently evaluating the combination of arsenic trioxide 0.25 mg/kg/day, 5 days/week for 2 weeks followed by 2 weeks with no therapy, thalidomide 100 mg/day p.o. and ascorbic acid 500 mg t.i.d. in patients with MDS. The initial report of 14 patients shows 7 HI (50% response rate) including 3 patients achieving transfusion independence. Combinations of arsenic trioxide with other agents including cytarabine and amifostine, are currently under investigation.

5. Expert opinion and conclusions

Arsenic trioxide is an old drug which has recently been rediscovered. The renewed attention on this compound has revealed its wide range of biological activities which support the rationale for its use in MDS, a disease for which no standard treatment has yet been established. The early results of the ongoing clinical studies confirm that arsenic trioxide has a favourable toxicity profile and can be administered on an out-patient basis in the MDS population which involves a majority of elderly patients. As previously reported in patients with APL, most of the adverse effects are non-chemotherapy-like, moderate and regress shortly after cessation of therapy.

The efficacy assessment arsenic trioxide in MDS still rely on preliminary reports. However, the results of the different ongoing Phase II studies are consistent and some conclusions can be drawn: arsenic trioxide has clinical activity in MDS; the overall response rate is in the 25 – 30% range; the main clinical benefit is represented by HIs which can be observed across all haematological lineages; durable transfusion-independence can be achieved whereas complete or partial remission are anecdotal. These results thus indicate that arsenic trioxide is a promising drug for the treatment of MDS. Its favourable toxicity profile encourage the design of combined treatment strategies.

The current data on arsenic trioxide also raise several questions. Firstly, the issue of the optimal dosing schedule is not yet solved. Initial studies used daily dosing at a fixed dose of 10 mg/day derived from the Chinese experience [3] and was quickly replaced by a weight-adapted dose of 0.15 mg/kg/day [5]. Based on pharmacokinetic data, sequential schedules have been designed. One of these which includes a 5-day loading dose followed by a twice-weekly maintenance treatment is used in the European MDS trial. Although validation of this scheme by pharmacokinetics is warranted, the preliminary results favour the use of this regimen. Its clinical efficacy is comparable to the '2 weeks on/2 weeks off' schedule which has been tested in a similar population and the tolerance seemed to be improved with notably the absence of QT prolongation recorded in the first 47 patients evaluated.

Another important issue is to determine if and which subgroups of MDS are more likely to respond to arsenic trioxide. MDS is a heterogeneous group of diseases and different

subgroups may show different response rates. For example, the cytogenetic categories identify several entities with variable prognostic which may well show specific sensitivity profiles to arsenic trioxide. This is also true for the various stages of the disease which have different biological features. Increased apoptosis is prominent in the early stages of the disease whereas cell cycle disturbances, RAS mutations or genomic instability predominate in the later stages [65]. The analyses conducted on the preliminary experience have not revealed a clinical or biological profile among the responders, but the data currently available are not mature enough to draw definitive conclusion. With large number of patients from the ongoing Phase II studies, such analyses will soon be possible. In addition, it is critical to the determine which of the many intra-cellular targets of arsenic trioxide operate in MDS. The complexity of the disease and the multiplicity of the mechanisms of action of the drug have made the identification of these targets difficult. With the developments of the DNA microarray technology, large scale genomic analysis become available and may provide new insights on the molecular effects of arsenic trioxide in MDS.

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